Screening for Neuroblastoma in France: Methodological Aspects and Preliminary Observations

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A pilot study of neuroblastoma mass screening was initiated in January 1990 in the Rhone French district. The expected number of births per year is 26,000. The study is designed for a 5-year period with three major goals: 1) measurement of the compliance rate of a voluntary test at 4 months of age; 2) evaluation of the technical value of high-pressure liquid chromatography (HPLC) as a screening method; and 3) detailed biological characterization of all detected tumors.

61,551 children were screened between May 1, 1990 and December 31, 1993. Participation was 69% in 1990, 81.5% in 1991, and over 83% in 1992.

HPLC was a satisfactory assay method. The number of clinical examinations required for positive tests as defined in the protocol is 1 per 3,621 tests. The false positive rate is 1 per 3,583 tests.

Eight neuroblastomas were discovered by-screening (one stage I, three stage II, one stage III, three stage IVs). All are alive and well but were good prognosis cases according to the main prognostic factors. Five patients were discovered before screening (so called Halo effect): one stage I, one stage III, three stage IVs. One died of disease and four are alive in complete remission after treatment. Two patients were false negative (one stage III with *N-myc* amplification, one stage IV with bad prognosis features) and three cases of neuroblastoma were missed because of noncompliance with the screening program.

This pilot study concludes on the feasibility of a mass screening program in France. The estimated cumulative incidence of neuroblastoma at 3 years is 1 per 4,375 living births and overdiagnosis is probable. All the detected cases were of good prognosis and the false negative ones were poor prognosis cases. **Med. Pediatr. Oncol. 28:81–91** © 1997 Wiley-Liss, Inc.

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INTRODUCTION

Neuroblastoma is a rare disease. Its annual incidence is 7–12.6/10^6 for children less than 15 years of age in developed countries. However, it accounts, in the United States (1981–1986 period), for an annual mortality of, respectively, 4.9/10^6 and 9.15/10^6 in the age groups 0–14 and 0–4 years. The overall survival rate at 10 years is 55%. The prognosis is age and stage dependent, and there is a significant correlation between age and stage at diagnosis. Thus, screening may permit detection of disease in an earlier nonsymptomatic stage (preclinical phase), and should decrease overall mortality [1,2].

Neuroblastoma screening satisfies the criteria required for effectiveness of a mass screening program in terms of the disease, the context, the population, and the test itself [3]. The benefit to be expected from a screening program is larger if the length of time spent in the preclinical phase (sojourn time) is longer, and if survival in its absence is poor. While survival of neuroblastoma patients is well documented, the length of the preclinical phase is not; screening program data may be helpful to obtain this information on this latter variable [4].

Screening programs try to detect a disease in its preclinical phase. However, recent data suggest that neuroblastoma is a heterogeneous disease, and that cases detected in their preclinical phase at screening are mainly those which would either show spontaneous regression or have good prognosis even if detected clinically [5]. In addition to stage and age at diagnosis, biological factors such as DNA content or ploidy, the presence of chromosome 1p abnormalities, and the number of *N-myc* oncogene copies are major prognostic factors [6]. These allow the distinction between two separate diseases: one occurring in young infants, with favorable prognostic biological factors and a high rate of long-term survival, the other occurring later and associated with early treatment failure. There is, however, no evidence that the former

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is the first step to the latter. Some studies suggest that *N-myc* amplification is constant throughout the course of the disease [7]. Thus, one of the major unsolved questions raised by ongoing screening programs is: Does early detection of children with poor prognosis features improve their prognosis? Moreover, it is suggested that a screening program may increase the incidence of good prognosis cases by detecting some tumors that would spontaneously regress and result in an overdiagnosis.

Following the Japanese experience of Sawada et al. in Kyoto and Takeda et al. in Sapporo [8,9], several groups implemented pilot studies on screening babies for neuroblastoma [10–12]. Since there was no population-based data showing reduced overall mortality from mass screening for neuroblastoma in Japan [13], all these groups had in mind to subsequently set up such studies to demonstrate an actual decrease in mortality rate.

Screening is based on the observation that 85–90% of neuroblastomas secrete either vanillylmandelic acid (VMA) and/or homovanillic acid (HVA) in detectable quantities in the urine at time of diagnosis [14,15]. The spot-test of La Brosse [16] was the first analytical procedure used in Japan as early as 1973. Recently, more reliable procedures have become available: thin-layer chromatography, gas chromatography-mass spectrometry, and enzyme immunoassay [17,18]. The high-pressure liquid chromatography with electrochemical detection (HPLC-ECD) avoids any extraction, purification, or transformation steps. The French and the German groups (Stuttgart and Hamburg) have chosen this procedure as it now probably offers the best cost/benefit ratio [10].

Considering the epidemiological data of the Rhone-Alpes region [19], we initiated a 5-year feasibility study (from January 1990 to December 1994) of a neuroblastoma mass screening for infants 4 months old in the Rhone department. The goals of this study were 1) to assess the rate of participation by parents in voluntary and free of charge screening, 2) to investigate HPLC as a methodological procedure for sensitivity and quality of the VMA and HVA measurements, and 3) to determine the biological characteristics of the neuroblastomas. Ancillary questions were also addressed through this program: 1) Would our results confirm the available epidemiological data from national and regional surveys? 2) What is the feasibility of a large-scale (26,000 births per year) cancer screening program for children in France?

After the first 3 years of the study, preliminary results and observations concerning the methodology are available, although it is still too early for any definitive evaluation. The rationale for early screening is still a subject of major debate [20], and these preliminary results must be considered to redefine the major objectives of a neuroblastoma mass screening program.

PATIENTS, MATERIALS, AND METHODS

Target and Control Populations: Definitions

From January 1990 to December 1992, 78,827 births were registered in the Rhone department (1.5 million inhabitants) which is part of the Rhone-Alpes area in France. The target population of the screening neuroblastoma pilot study includes each child born in the Rhone department during the 5-year period (1990–1994). A birth registry of metabolic diseases screening in the same area was used to check compliance.

A case is defined as a neuroblastoma diagnosed in a child from the target population. A screened case is a neuroblastoma diagnosed by the screening procedures (tests and diagnosis procedures) performed before 1 year of age. A missed case is a child with neuroblastoma who did not participate in the screening program before 1 year of age. A false negative is a neuroblastoma with a negative test or diagnosis procedures performed previously whatever the age at diagnosis. A false positive is defined as two consecutive positive tests with negative clinical and radiological findings. An early case is a neuroblastoma diagnosed before 4 months of age.

The French Society of Pediatric Oncology (S.F.O.P.) network is part of the project and notification of any diagnosis of neuroblastoma in a child born in the Rhone department is sent to the Center for Neuroblastoma Screening. The nearby region of Auvergne and three other French regions are used as controls for incidence and survival rates through childhood malignancies population-based registries.

Results are based on birth cohort calculations, which means that a child born in 1991 belongs to the 1991 cohort whatever the date of the diagnosis. Incidence and false negative rates are expressed as the number of cases (neuroblastoma or neuroblastoma with negative screening) in the target population at time of analysis. The cumulative rates at 3 years are estimated by using actuarial methods.

Characteristics of Neuroblastoma Screening

The test is performed from 4 months of age to 1 year on a voluntary basis. The participation is free of charge for parents. The biological technique used is HPLC (see below). Major sponsorship came from the National Social Security research funds.

Information and Recall Procedures

Information on the screening program and the kit suggesting participation are inserted in the carnet de sante (a specific book record of health problems delivered at birth to each French child). Information is essentially given in the maternity hospitals by nurses and pediatricians. A videotape is also available to each mother during her stay in the hospital. During the first months of life,

systematic visits by the pediatricians are opportunities for further advice. The date of the theoretical screening test is highlighted in this book record.

For noncompliant families (test not sent when the child reaches 4 months of age), a recall letter is sent at 6 and if necessary at 8 months. A mailing is systematically sent annually to each pediatrician in the area to explain the objectives of the program and to show results. Moreover, advertising campaigns are conducted to sensitize the population.

Urine Collection

The urine sample is collected on a rectangular (56 × 30 mm) Whatman paper 17CHR, previously cut into 7.8 × 20 mm tongues. Each tongue can absorb as much as 140 μl of urine. The urine may be collected in several different ways: either soaking the paper in liquid urine, inserting it in the urinary stream (best procedure), or inserting it between the skin and the napkin. Then the paper is air dried, sheltered from sunlight, and sent by post. When the sample is received, an ultraviolet examination is performed to confirm that urine has well impregnated the paper (if not, a new test is requested from the parents).

VMA, HVA, and Creatinine (Cr) Measurements

The paper tongue is agitated for 3 minutes in a 1.4 ml aliquot of elution liquid (phosphate buffer) which provides, after centrifugation, an approximate 1/10 dilution. Cr is assessed by the kinetic method with Technicon RAXT.

The sample (eluate) is diluted fivefold (final dilution approximately 1 to 50) and directly analyzed by HPLC without further manipulations. One complete analysis is obtained in less than 10 minutes with a mobile phase containing an ion-pairing reagent and a reverse-phase column (Bio-Rad Laboratories). Calibration with a standard solution of VMA and HVA is scheduled after each series of 10 samples. If a Cr value of less than 34 mg/l (300 μmol/l) is observed, a second sample is then requested.

The Center for Screening of Neuroblastoma is a participant in the French national control quality program for creatinine, VMA, and HVA assays.

Procedures for Positive Tests

In case of elevated catecholamine results, the Screening Center is in charge of requesting a control to parents. In case of elevated levels in this control, parents are advised to come for consultation with a general pediatrician from the University Pediatric Hospital.

Clinical examination, abdominal ultrasonography, and chest X-ray are performed. If all these investigations are negative, the child is sent back home and further urine tests are requested, until negative results are obtained (after a maximum 2-month period). In the case of an abnormality in any of these investigations, the child is referred to the pediatric oncology team at the Centre Leon Berard. Complete staging is then performed, including a computed tomographic (CT) scan of the abdomen and/or lungs, urine and catecholamine levels over 24 hours, full blood cell count, electrolytes, lactate dehydrogenase (LDH), ferritin, Neuron Specific Enolase (NSE) in the serum sample, and meta-iodobenzylguanidine (MIBG) scanning. In case of bone positivity, technetium scanning and skeleton X-rays are performed. Under general anesthesia, a bone marrow smear from each anterior and posterior iliac crest is performed. When feasible (usually infants more than 8 months old), one bone marrow biopsy of each iliac crest is obtained. Neuroblastomas were classified according to the Shimada classification [21]. The *N-myc* amplification was analyzed by using the Southern blot method and chromosome 1 by the fluorescein in situ hybridization (FISH) method as previously reported [22]. Ploidy was not performed.

Treatment of Cases

The treatment is adapted to the staging. For localized operable tumors, immediate surgery is advised. For nonoperable localized tumors, the child is included in the French national NBL90 study, i.e., two courses of etoposide + carboplatin followed by two courses of CADO (cyclophosphamide, Adriamycin, vincristine), followed by surgery. Children with IVs disease are also included in the NBL90 study which includes either a watch and wait attitude if no evident progressive disease is detected, or otherwise chemotherapy using low-dose cyclophosphamide and vincristine (CO). The children with stage IV disease are treated according to *N-myc* amplification in the tumor. When no *N-myc* amplification is detected, they receive treatment with CADO and etoposide-carboplatin. In case of *N-myc* amplification, they are treated with the more intensive NB87-LMCE3 protocol as published previously [23]. Follow-up of children is performed every 2 months following termination of therapy.

RESULTS

Normal and Upper Limit (Cut-Off) Values

61,663 screening tests were performed during this 3-year feasibility study. The calculation of normal values for HVA and VMA is based on the totality of our results (initial and control measurements). Three standard deviations (SD) were chosen as the upper limit value for detection. The means (M) and cut-off values are expressed as microgram per milligram of Cr. Results are as follows: for VMA, M = 9.8 μg/mg, SD = 4.8 μg/mg, cut-off = 24.2 μg/mg; for HVA, M = 16.7 μg/mg, SD = 5.4 μg/mg, and cut-off = 33.1 μg/mg.

TABLE I. Results on Neuroblastoma Mass Screening in the Rhone District From January 1, 1990 to December 31, 1992

	Year of birth			
	1990	1991	1992	Total
No. of births	26,268	26,460	26,043	78,771
Screened children	18,168	21,578	21,805	61,551
Participation rate	69.16	81.55	83.73	78.14
Clinical examinations	3	15	7	25
False positive rate	1/9,084	1/1,541	1/21,805	1/3,621
(no. disease free/no. screened)	(2/18,168)	(14/21,578)	(1/21,805)	(17/61,551)
Screened cases	1	1	6	8
Late screening	0	0	1	1
Missed cases	0	2	0	2
Early cases	2	1	2	5
(before 4 months)				2
False negative	1	1	0	18
Cases	4	5	9	1/4,376
Incidence rate	1/6,567	1/5,292	1/2,895	1/4,259[a]

[a]Estimated cumulative incidence at 3 years (actuarial method).

Participation and Results

From May 1, 1990 to December 31, 1993, 61,551 children born between 1990 and 1992 participated in this screening program. Participation rates are, respectively, 69.16% (18,168 of 26,268 children), 81.55% (21,578 of 26,460 children), and 83.73% (21,805 of 26,043 children) for the 1990, 1991, and 1992 birth cohorts. A technical problem due to a delay in the insertion of the kits in health records partly explains the low compliance rate in 1990. The delay to participate is now shorter than at the beginning of the program and more than 95% of the participating population is screened before 6 months of age (i.e., before the first recall procedure) compared to 85% in 1990. A second sample was requested in 5% of received tests because of a low level of Cr or an inadequate urine collection.

Of the 78,771 children in the target population, 8 cases of neuroblastoma have been detected by the screening program (screened cases). In addition, five cases were discovered before the age of 4 months (halo effect). Two cases were missed because the parents did not perform the test (the ages at diagnosis were 6 and 20 months, respectively) and one case participated after 12 months of age (late screening) and was considered as missed. In two children with negative tests, a secreting neuroblastoma was discovered later at 10 months and 2 years (false negative cases). Overall incidence rate is 1 case per 4,376 living births. Results according to birth cohort are shown in Table I. The estimated cumulative incidence of neuroblastoma at 3 years is 1 per 4,259 children.

Screened Cases

Three of eight were stage IVs (with involvement of liver [two], skin [one], bone fixation at MIBG scanning [one], positivity of marrow immunology [one], one was stage I, three were stage II, and one was stage III (Table II). Shimada classification was favorable in six screened cases, unfavorable in one IVs patient, and was not done in one stage III who required initial chemotherapy. *N-myc* amplification was absent in all screened cases. Ferritin was raised in one of eight cases (373 mg/l, stage IVs), LDH in four of eight cases (704–1,038 UI/l), and NSE in all screened cases (22–72 μg/l). Two of eight required chemotherapy (one IVs received eight CO courses for clinical evolution after initial surgery, and one stage III). All underwent local surgery. All are alive and free of disease 2–27 months post diagnosis (median = 12 months).

One child had a later screening (13 months) and is not considered as a screened case (Table III). He has a stage III with favorable Shimada classification, although Joshi grade II due to age. Biological features were favorable. He is currently under chemotherapy by the NBL90 protocol after an incomplete removal of his mass.

Halo Effect

During the same period, there was an apparent increase of neuroblastoma incidence, since five cases were diagnosed before screening (Table IV). Two patients with IVs neuroblastoma were diagnosed neonatally: one had a fatal course due to abdominal progression despite chemotherapy (CO) and local radiation therapy. Since no surgery or autopsy could be performed, there is no information on Shimada or *N-myc* amplification. Ferritin level was elevated. The second infant underwent initial surgery with subtotal removal and nephrectomy. Shimada was favorable, and there was no *N-myc* amplification. He required chemotherapy (NBL90) for disease progression, but is currently in complete remission of liver and skin metastases 2 months off therapy. Three other infants were diag-

TABLE II. Neuroblastomas Diagnosed by Screening*

Patient no. Birth date	VMA-HVA 1st sample control (delay)	Date of diagnosis Age at screening	Stage	Shimada	*N-myc* deletion of chromosome 1	NSE (μg/l) (N = 12 μg/l)	LDH (UI/l) (N < 500 UI/l)	Ferritin (μg/l) (N < 100 μg/l)	Treatment	Survival (from diagnosis)
3 Nov 2, 1990	109-62 118-55 (11 days)	March 22, 1991 4 months	IVs	Favorable	NA No	50	1,038	42	Surgery + 8 CO	33+
8 Dec 16, 1991	53-63 59-74 (15 days)	June 23, 1992 4 months, 2 weeks	IVs	Unfavorable	NA Yes	22	706	76	Surgery	18+
10 March 3, 1992	36-33 35-38 (13 days)	Aug 8, 1992 4 months, 2 weeks	II	Favorable	NA No	ND	415	35	Surgery	17+
9 Jan 15, 1992	55-40 10-41 (14 days)	Aug 5, 1992 5 months	I	Favorable	NA No	72	797	4.5	Surgery	17+
12 April 16, 1992	44-35 52-45 (29 days)	Oct 26, 1992 4 months, 2 weeks	IVs	Favorable	NA No	43	495	373	Surgery	14+
14 Aug 14, 1992	50-26 80-37 (32 days)	Feb 18, 1993 4 months, 3 weeks	III	ND	NA No	35	704	13	Chemotherapy + surgery	10+
17 Nov 25, 1992	90-164 82-117 (12 days)	March 20, 1993 4 months	II	Favorable	NA No	35	535	40.8	Surgery	8+
15 Dec 28, 1992	50-114 42-86 (8 days)	March 3, 1993 4 months	II	Favorable	NA No	20	546	96	Surgery	8+

*VMA, N < 24 μg/mg creatinine; HVA, N < 33 μg/mg creatinine. ND, not done; NA, not amplified.

TABLE III. Neuroblastomas Diagnosed by Late Screening (> 12 Months)*

Patient no. Birth date	VMA-HVA 1st sample control (delay)	Date of diagnosis Age at screening	Stage	Shimada	*N-myc* deletion of chromosome 1	NSE (μg/l) (N = 12 μg/l)	LDH (UI/l) (N < 500 UI/l)	Ferritin (μg/l) (N < 100 μg/l)	Treatment	Survival (from diagnosis)
18 Jan 3, 1992	613-17 323-185 (7 days)	March 25, 1993 13 months	III	Favorable	NA No	67	679	5	Chemotherapy	9+

*ND, not done; NA, not amplified.

TABLE IV. Neuroblastomas Diagnosed Before Screening (i.e., Before 4 Months of Age)*

Patient no. Birth date	VMA-HVA	Date of diagnosis Age at screening	Stage	Shimada	*N-myc* deletion of chromosome 1	NSE (μg/l) (N = 12 μg/l)	LDH (UI/l) (N < 500 UI/l)	Ferritin (μg/l) (N < 100 μg/l)	Treatment	Survival (from diagnosis)
1 Feb 5, 1990	159-46	Feb 6, 1990 Neonatal	IVs	ND	ND ND	ND		325	Chemotherapy + RT	Death at 20 days
2 June 20, 1990	139-55	Aug 20, 1990 2 months	III	ND	ND ND	ND	740	145	Surgery Chemotherapy Surgery + peroperative RT RT	40+
5 Sept 16, 1991	16-13 (dopamine increased)	Oct 15, 1991 1 month	I	Favorable	NA No	7.6	576	51	Surgery	26+
7 Feb 12, 1992	28-25	May 25, 1992 3 months, 3 weeks	IVs	Favorable	NA No	9.5	500	120	No	19+
11 Sept 7, 1992	331-67	Sept 8, 1992 Neonatal	IVs	Favorable	NA No	200	2,800	400	Surgery Chemotherapy	17+

*ND, not done; NA, not amplified; RT, radiation therapy.

nosed at 1, 2, and 4 months of age. Two of them (stages I and IVs) had favorable Shimada, without *N-myc* amplification, and had favorable biological features; they required only local surgery. The last one is a 2-month old girl with a stage III neuroblastoma at diagnosis. The surgery was delayed (7 months post diagnosis) but was unsuccessful. She thus required six courses of chemotherapy (NBL90), before an incomplete surgery associated with intraoperative radiation therapy. She is alive in complete remission 23 months after completion of treatment.

Missed Cases

In two children, no screening was performed (Table V). One stage III neuroblastoma was discovered at 6 months of age; no Shimada classification is available due to prior chemotherapy, and there is apparently no *N-myc* amplification although few cells were available for investigation. She had a normal ferritin level but her LDH level was elevated (2,600 UI/l), and she received NBL90 chemotherapy. She is alive and well, off treatment. The other case is a 20-month old boy with poor prognosis stage IV. He has no *N-myc* amplification, but poor prognostic biological and clinical (poor response to induction therapy with persistent skeleton lesions) features. This boy is alive in partial response.

False Negative Cases

Screening was negative in two cases (Table VI). One girl had a negative screening at 4 months and developed an explosive *N-myc* amplified stage III abdominal tumor with poor prognostic biological features at 10 months. She was treated with aggressive chemotherapy, and underwent microscopically complete surgical removal and intraoperative radiotherapy. She relapsed 2 months after completion of treatment, responded transiently to second-line therapy, received bone marrow transplantation in progressive disease, and ultimately died of disease. The other patient had a negative screening test at 4 months of age. He developed a stage IV abdominal neuroblastoma diagnosed at 18 months of age. The biological criteria showed poor prognostic features (ferritin = 145 μg/l, LDH = 1,522 UI/l, NSE = 300 μg/l). Neither Shimada classification (surgery performed after induction therapy) nor *N-myc* amplification (technical problem during the manipulation of the fine needle biopsy specimen) is available. He received the NB87-LMCE3 protocol and is currently in partial remission after an autograft.

The case of one other child should be outlined. He underwent a bilateral uronephrectomy with kidney transplantation for an incomplete prune belly syndrome. The left adrenal gland was considered as suspect and removed. Histologically, it contained few foci of calcifications, with some mature ganglionic cells. It may be interpreted either as a post hemorragic lesion or as a regressing neuroblastoma. In our view, there is no sufficient evidence

TABLE V. Missed Cases of Neuroblastomas (i.e., not Done by the Parents)*

Patient no. Birth date	VMA-HVA	Date of diagnosis Age at diagnosis	Stage	Shimada	*N-myc* deletion of chromosome 1	NSE (μg/l) (N = 12 μg/l)	LDH (UI/l) (N < 500 UI/l)	Ferritin (μg/l) (N < 100 μg/l)	Treatment	Survival (from diagnosis)
4 Jan 7, 1991	786-1,318	July 15, 1991 6 months, 1 week	III	ND	NA No	ND	2,600	65	Chemotherapy Surgery	29+
16 June 21, 1991	708-433	March 5, 1993 20 months, 2 weeks	IV	ND	NA No	131	1,430	200	Chemotherapy Surgery ABMT	Alive in PR1 10+

*VMA, N < 24 μg/mg creatinine; HVA, N < 33 μg/mg creatinine. ND, not done; NA, not amplified; Autologous Bone Marrow Transplantation (ABMT).

TABLE VI. False Negative Cases (i.e., Negative Test at Time of Screening)*

Patient no. Birth date	VMA-HVA screening control diagnosis	Date of diagnosis Age at diagnosis Age at screening	Stage	Shimada	*N-myc* deletion of chromosome 1	NSE (μg/l) (N = 12 μg/l)	LDH (UI/l) (N < 500 UI/l)	Ferritin (μg/l) (N < 100 μg/l)	Treatment	Survival (from diagnosis)
13	17-23	Nov 12, 1992	IV	ND	ND	300	1,520	145	Chemotherapy	13+
May 14, 1990		2 years, 6 months			No				Surgery	
		6 months							ABMT	
	241-196									
6	23-29	May 15, 1992	III	Favorable	Amplified	596	11,350	470	Chemotherapy	Death at 1 month
July 1991	8-22	10 months, 1 week			(50)				Surgery	
	37–117	4 months, 3 weeks			ND				RT	
									Relapse:	
									Chemotherapy	
									ABMT	

*VMA, N < 24 μg/mg creatinine; HVA, N < 33 μg/mg creatinine; ND, not done; NA, not amplified.

to include this child in our cohort, though it may be a consequence of the halo effect.

False Positive Tests

As shown in Table I, the number of clinical examinations required for positive testing varied from 1 per 21,805 performed tests in 1990 to 1 per 1,541 performed tests in 1991. In 1992, the cut-off value for HVA was slightly increased from 30.1 μg/mg of Cr to 33.3 in order to reduce the number of false positive tests. Seventeen clinical examinations out of 61,551 screened children were not followed by a positive diagnosis of neuroblastoma. The mean false positive rate of this screening program is then 1 per 3,621 tests.

DISCUSSION

The French experience in the Rhône district shows that the compliance rate for the 4 months of screening is very good, being constantly over 80% after the initial period. Moreover, more than 95% of tests are spontaneously sent before the first recall procedure which shows a good sensitivisation of parents and pediatricians.

The HPLC gives satisfactory results with the cut-off values that have been defined and adjusted. The high rate of false positives during 1991 is probably due to dietary factors. There have been no technical problems with the two machines that are permanently running in the Neuroblastoma Screening Laboratory.

During the screening period, the incidence increased to 1 per 4,375 living births compared to 1 per 8,430 living births observed in our previous study in the same area [19]. Two hypotheses may be raised: halo effect and overdiagnosis. A halo effect may be noticed, with an increased detection rate for children less than 4 months old. This effect is probably due to an increased awareness of the physicians and pathologists of the Rhone department. It remains to be seen if this halo effect will be detected in the nearby departments and regions.

An overdiagnosis must be suspected, considering the increased detection rate of good prognosis cases (absence of *N-myc* amplification, low ferritin and LDH levels, and favorable Shimada classification in all detected cases). It has been calculated that the incidence of in situ tumors is 40–50 times higher than the incidence of neuroblastoma detected clinically, and most of those tumors disappear spontaneously [14]. Nevertheless, these findings must be confirmed since in the Auvergne region, which is our control region, the incidence (1986–1991) is 1 per 4,593 living births (unpublished data).

Since the nationwide screening was initiated in Japan, more than half a million infants have been screened, and 598 cases have been detected [24]. The incidence rate varies with screening method from 1 per 20,000 with the qualitative VMA spot-test to 1 per 5,000 with HPLC. The incidence increased from 4.5 to 8.39 cases per million

children per year, showing clearly that the screening program induces a noticeable overdiagnosis. There is a significant shift of the age of diagnosis with more children 1–2 years of age, less children between the ages of 2 and 3 years, but no change in children over 4 years of age. Finally, the ratio of cases detected under 1 year of age increased from 28% in 1980 to 61% in 1989 [25].

In Sapporo City, the annual incidence of advanced neuroblastoma was significantly reduced during the 1981–1989 screening period (1.1 per 100,000) as compared with the prescreening period (3 per 100,000). However, contrary to all other studies, there is no significant increase in the overall incidence of neuroblastoma between the two periods, suggesting a bias in the report. The experience in Sapporo City [26], with HPLC screening performed at 6 months of age since 1981, shows a significant decrease in the proportion of infants diagnosed over 1 year of age, and diagnosed as advanced stage in the screened population, as compared with those not screened. However, the latter group is small and heterogeneous (half detected before 1979), precluding any final conclusion.

In Saitama, the screening program was performed at 6 months of age and detected cases with favorable biological prognostic factors (lack of *N-myc* amplification, and hyperploid or near-triploid karyotypes) and excellent survival rates. It was suggested that the tumors with near-diploid or tetraploid karyotypes, often accompanied by *N-myc* amplification, grew after the time of screening. Either those tumors had an explosive multiplication rate, or they were too small at the time of screening to be diagnosed. All these findings lead the authors to propose two successive screening programs (at 6 and 12 months) [27].

In our experience, all detected cases had favorable prognostic features and the missed cases had poor prognostic features. The delay between the negative screening and detection was particularly short for the first case (5 months), suggesting that this type of highly proliferative tumor, with amplified *N-myc*, elevated LDH and ferritin level, may be particularly difficult to detect, since it became evident clinically at 10 months of age, and would thus also have escaped screening performed at 12 months old.

The reported clinical results obtained with screening seemed impressive at first glance, since 75% of the detected children are in the good prognosis group (I, II, IVs) and the overall survival is 97%. However, there is no convincing evidence that decreased mortality has been reached in Japan, since there were no reliable data of mortality in Japan prior to screening. Thus, in the absence of a control region, there is no way to assess the rate of overdiagnosis (i.e., tumors that would have spontaneously disappeared in the absence of diagnosis) induced by screening and to show a decrease in the mortality rate [17,28].

The Japanese national mortality data obtained retrospectively through review of death certificates suggest a significant decrease in annual death rate for the 1–4-year-old age group only (from 1.3 to $0.6/10^5$ children 1–4 years old between 1979 and 1987) [29]. The 67% 5-year survival obtained during the screening period is not superior to most published series in the United States (55%) and Germany (50%) [30,31]. Moreover, the Finnish experience, with an unselected nationwide population-based registry and complete follow-up shows that the 5-year overall survival rate has increased from 24% in the 1960–1969 period, to 57% in the 1980–1986 period, suggesting that advances in clinical diagnosis and treatment are responsible for this increase [20]. Stiller [32] recently published similar data from the Great Britain registry where incidence has increased by 36% and mortality decreased by 27% from the 1971 to 1975 period to the 1981–1985 period. Thus, historical controls are not useful for comparison. The survival rate in the Kyoto region showed an increase from 17% in the 1962–1974 prescreening period to 71% in the 1974–1988 screening period [33]. Similarly, the total number of deaths due to neuroblastoma in Sapporo City decreased from 1.3 per year during the screening as compared to 3.3 per year during the prescreening period, and this was only significant in the 0–4-year-old group. However, as noted by Cole et al. [34], it is unlikely that this lower mortality rate might be explained by screening since the accumulated person-years by the screened population was still too low when the decrease was observed. In addition, it is possible that the mortality rates before 1975 were slightly overestimated in Japan, given the method of data collection and the high mortality rate of the 1–4-year-old group before 1985.

Based on these findings, a North American group initiated a controlled study aimed at comparing the mortality from all children diagnosed (either by screening or clinically) to that of several concurrent population-based non-screened control groups. The screening includes a two-step procedure with the first screening at 3 weeks and the second at 6 months. Preliminary reports [35,36] confirm that screened cases always have good prognostic biological features. No clear conclusion may yet be drawn concerning mortality rates from this program which will include 450,000 children.

A feasibility study in Newcastle suggests that the discrepancy between the observed and the expected number of cases gives further credence to the need for a statistically sound evaluative study to be carried out, with death from neuroblastoma as the only end point in the screened population. This study would require up to a million screened children and a well-matched unscreened population [12]. A meeting of statisticians recently held in Lyon suggested that the required sample size to confirm the utility of screening may be more than 2 million children (unpublished data).

CONCLUSIONS

Based on these data, the early screening (4 to 6 months) is feasible in France and the high participation rate observed in this program is sufficient to ensure the potential efficiency of a large-scale neuroblastoma mass screening. Nevertheless, a probable overdiagnosis must be suspected, considering the estimated incidence of neuroblastoma and the prognosis features of screened patients. These preliminary findings must be confirmed when the data of this 5-year pilot study are available with a 2-year follow-up.

In order to reduce this overdiagnosis of good prognostic tumor and not to detect spontaneously regressive tumors, an alternative proposal would be to screen much later after 1 year of age. Two years of age seems to be the upper limit since it is suggested that children less than 2 years old may have a better prognosis.

Screening at 12 months would seem to be the best age for a neuroblastoma mass screening. It would reduce the probable early overdiagnosis and may improve the overall survival rate (the 3-year overall survival rate of children less than 1 year old at diagnosis is 83%) [37]. A European group, including French, German, Austrian, English, and perhaps Norwegian and Danish participants, is discussing the feasibility of such a study.

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